Ph.D. Thesis

Receptor functions and genetic polymorphisms in schizophrenia

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Journal articles related to the thesis:

Kéri S, Antal A, Szekeres G, Benedek G, Janka Z: Visual information processing in patients with schizophrenia: evidence for the impairment of central mechanisms. *Neurosci Lett* 2000; 293:69-71. **IF: 2.091**

Kéri S, Antal A, Szekeres G, Benedek G, Janka Z: Spatiotemporal visual processing in schizophrenia. *J Neuropsychiatry Clin Neurosci* 2002; 14:190-196. **IF: 2.212**

Kéri S, Antal A, Szekeres G, Szendi I, Kovács Z, Benedek G, Janka Z: [Testing basic visual functions in the evaluation of extrapyramidal side effects of antipsychotic agents]. *Orv Hetil* 1998; 139:235-238.

Szekeres G, Janka Z: [Receptor polymorphisms and response to treatment in schizophrenia]. *Orv Hetil* 2002; 143:2027-2033.

Szekeres G, Pávics L, Janka Z: [Investigation of dopamine dysregulation hypothesis of schizophrenia with neuroimaging techniques]. *Ideggyógy Szle/Clin Neurosci* 2002; 55:226-232.

Szekeres G, Juhász A, Kéri S, Rimanóczy Á, Szendi I, Szabó Z, Janka Z: [Relationship between the efficacy of atypical antipsychotics and polymorphism of dopamine D3 receptor in schizophrenia]. *Ideggyógy Szle/Clin Neurosci* 2002; 55:377-381.

Szekeres G, Kéri S, Juhász A, Rimanóczy Á, Janka Z: The C270T polymorphism of the brain-derived neurotrophic factor (BDNF) gene is associated with schizophrenia. *Schizophr Res* 2003; 1879:1– 4. **IF(2002): 3.203**

Szekeres G, Kéri S, Juhász A, Rimanóczy Á, Szendi I, Cimmer C, Janka Z: The role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. *Am J Med Genet* (in press) **IF(2002): 2.334**

Pávics L, Szekeres G, Ambrus E, Kéri S, Kovács Z, Árgyelán M, Kanyó B, Csernay L, Janka Z: The prognostic value of dopamine receptor occupancy by ^{123}I-IBZM SPECT in schizophrenic patients treated with quetiapine. (submitted) *Eur Neuropsychopharmacol*

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Pávics L, Szekeres G, Ambrus E, Juhász A, Kéri S, Kovács Z, Galuska L, Janka Z: Dopamine D2 receptor SPECT in schizophrenic patients treated by quetiapine. *Eur J Nucl Med* 2001; 1034. **IF: 3.464**

Szekeres G, Pávics L, Antal A, Kéri S, Ambrus E, Kovács Z, Janka Z: Striatal dopaminergic blockade and visual contrast sensitivity in patients with schizophrenia treated with quetiapine. *Eur Neuropsychopharmacol* 2001; 11(Suppl. 3):S246. **IF: 2.437**

Szekeres G, Pávics L, Antal A, Kéri S, Ambrus E, Kovács Z, Janka Z: Integrative application of IBZM SPECT and psychophysical methods in the evaluation of dopamine receptor blockade in patients with schizophrenia. *Int J Neuropsychopharmacol* 2002; 3(suppl.1):S73. **IF: 3.341**

Janka Z, Szekeres G, Juhász A, Rimanóczy Á, Cimmer C, Szendi I, Kéri S: Relationship between dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphisms and therapeutic response in schizophrenic patients. *Int J Neuropsychopharmacol* 2002; 3(suppl.1):S113. **IF: 3.341**

Juhász A, Rimanóczy Á, Kéri S, Szekeres G, Janka Z: Brain-derived neurotrophic factor polymorphism in patients with schizophrenia. *Eur Neuropsychopharmacol* 2003; 13(Suppl. 4):S438. **IF(2002): 2.492**

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List of abbreviations:

^{123}I-IBZM SPECT	^{123}I-iodobenzamide single photon emission computer tomography
5-HT	serotonin
A	adenine
AIMS	Abnormal Involuntary Movements Scale
ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
C	cytosine
COMT	catechol-*O*-methyltransferase
DA	dopamine
DAT	dopamine transporter
DLPFC	dorsolateral prefrontal cortex
DNA	deoxyribonucleic acid
DRD2	dopamine D2 receptor
DRD3	dopamine D3 receptor
EPS	extrapyramidal side effects
G	guanine
GAF	Global Assessment of Functioning
Gly	glycine
Met	methionine
MINI	Mini International Neuropsychiatric Interview
mRNA	messenger ribonucleic acid
PANSS	Positive and Negative Syndrome Scale
PCR	polymerase chain reaction
PET	positron emission tomography
ROI	region of interest
S/O	striatum/occipital lobe occupancy ratio
SAS	Simpson Angus Scale
SD	standard deviation
Ser	serine
T	thymine
UV	ultra-violet
Val	valine
VCS	visual contrast sensitivity
VNTR	variable number of tandem repeats
WCST	Wisconsin Card Sorting Test

Contents

1. General summary

Schizophrenia is a chronic, devastating mental illness, affecting approximately 1 % of the average population around the world. Research on etiology, symptomatology, and therapeutic possibilities has gone through a revolutionary change due to modern neuroimaging techniques and molecular genetic methods.

The most known neurochemical model of schizophrenia is the dopamine (DA) theory (Carlsson & Lindqvist, 1963), which had an essential contribution to innovative trends in the development of new antipsychotics. Experiences coming from treatment with atypical antipsychotics, which are equally effective as compared to conventional neuroleptics but cause much less extrapyramidal side effects (EPS), contribute to the continuous modernization of the classical DA hypothesis. According to the revised DA theory, the most persistent cognitive deficit and the negative symptoms are related to the hypoactivity of the dorsolateral prefrontal cortex (DLPFC), while the positive symptoms during acute episodes are related to the hyperactivity of the ventral striatal elements of the DA system (Lieberman et al., 1997). The two disturbances are in causal relationship by means of extensive dopaminergic and glutamatergic connections between these structures (Weinberger et al., 2001). In the course of schizophrenia, more chronic recurrences of intermittent sensitized states of the subcortical DA system may occur. In these sensitive states, dopaminergic neurons are hyperresponsive to environmental stimuli and exposure to even moderate levels of stress, which are eventually associated with excessive DA release in the ventral striatum (n. accumbens) (Laruelle, 2000). This phenomenon is observable during active phases of the illness, but not in stable remission. Thus, the hyperdopaminergic state is phase-dependent, while "hypofrontality" is more persistent.

Trends in schizophrenia research include three new directions regarding the dopamine hypothesis: (i) relationship between cognitive impairments and DA dysfunctions, (ii) relationship between DA receptors as evaluated by neuroimaging methods and clinical/cognitive parameters, (iii) the role of genetic variants of molecules of the DA system (*e.g.* receptors and transporters) in clinical symptoms, cognitive performance, and therapeutic response. These general issues were targeted by this thesis.

One of the most fundamental functions of the human visual system is that we are able to discriminate stimuli from their background using luminance differences: the contrast between the stimulus and its background. Visual contrast sensitivity (VCS) functions are

modulated by DA (Masson et al., 1993). In hypodopaminergic states evolved from different origins, contrast sensitivity is reduced at low and medium spatial frequencies (Bodis-Wollner et al., 1987; Kéri et al., 1998), which suggests that the measurement of VCS as a peripheral, non-invasive method seems to be a useful tool to detect the state of the DA system. In our first experiment, we tested the effect of the high and the low dose of quetiapine, an atypical antipsychotic, on DA system by using three independent methods: (i) ^{123}I-iodobenzamide single photon emission computer tomography (^{123}I-IBZM SPECT) (ii) clinical symptom scales, and (iii) VCS. In addition, we identified a subgroup of clinically stable patients with high risk of relapse in the course of a six months follow-up period. All of the three methods confirmed that quetiapine has low antidopaminergic potential even in the high-dose condition. The patients with decreased striatal ^{123}I-IBZM uptake showed worsening of psychotic symptoms during the follow-up period. We hypothesize that it was caused by the persisting hyperactivity of the striatal dopaminergic system even after apparent clinically improvement.

The heritability of schizophrenia is well illustrated by a higher risk of morbidity among biological relatives of patients as compared to the general population. The risk ratio for schizophrenia relatives correlates with the degree of relationship (Tsuang, 2000). Association and linkage studies intended to find out the susceptibility genes excluded the possibility of monogenic forms of inheritance. The transmission is attributable to complex polygenic factors. Neuropharmacological and neurochemical investigations demonstrating several anomalies in the monoamine neurotransmission, particularly for the DA and serotonin (5-HT) systems, have stimulated a series of association studies. The relationship between schizophrenia and the dopamine D3 receptor (DRD3) gene Ser9Gly polymorphism has previously been demonstrated (Dubertret et al., 1998). Changes in the amino acid sequence could modify the function of the receptor, which may result in critical changes in the clinical phenotype such as symptoms and cognitive profile and response to antipsychotic medication. The more frequent occurrence of Ser9 allele in non-responder groups of schizophrenic patients has been published earlier (Shaikh et al., 1996; Scharfetter et al., 1999), which has not been confirmed by Malhotra and coworkers (1998). In our second study, we investigated the relationship between DRD3 gene Ser9Gly polymorphism, cognitive functions and response to atypical antipsychotic medication. Our results suggest that more severe impairments in prefrontal executive functions and worse therapeutic response are associated with the Ser9Ser genotype and the Ser9 allele.

According to the neurodevelopmental hypothesis of schizophrenia, genetic and/or environmental factors disrupt early central nervous system development, producing long-term

vulnerability to normal developmental processes (*e.g.* synaptic pruning during preadolescence) and to stress events that then lead to manifest psychotic symptoms (Maynard et al., 2001). Normal development, migration, differentiation and survival of neuronal cells, including dopaminergic neurons, depend on the timed activation of trophic factors that are genetically determined (Guillin et al., 2001). An important representative of these factors is the brain-derived neurotrophic factor (BDNF), which has multiple roles in developmental processes (Webster et al., 2002). In addition, BDNF specifically influences the expression of dopamine D3 receptors, which may link the dopamine and neurodevelopmental theories of schizophrenia. Therefore, it is plausible to hypothesize, that genetic variants of BDNF and schizophrenia may be associated. We sought the occurrence of a recently found single nucleotide substitution (C270T) polymorphism of BDNF in schizophrenia (Kunugi et al., 2001). The mutated type T allele and the C270T genotype were significantly more frequent in the patient (13.9 % and 25.7 %) compared with the controls (2.9 % and 5.9 %), suggesting that the T allele may contribute to susceptibility to schizophrenia. Table 1 shows overview of experiments.

Table 1. Overview of experiments underlying the thesis

Investigation of striatal dopamine functions	^{123}I-IBZM SPECT	
	Measure of visual contrast sensitivity	
	Clinical examinations	
Investigation of genetic effects	DRD3 Ser9Gly & DAT 9/10 VNTR polymorphism	association analysis
		cognitive performance
		response to treatment
	BDNF C270T polymorphism	association analysis

2. Introduction

2.1. Investigation of receptor functions and antipsychotic actions

2.1.1. Neuroimaging techniques

The classical DA hypothesis of schizophrenia suggests that the hyperactivity of the DA system is responsible for the psychotic symptoms of the disease (Carlsson & Lindqvist, 1963). The initial concept was based on indirect observations. The principle has risen from the facts that agonists of dopamine D2 receptor (DRD2) may provoke psychotic symptoms (Angrist & Gershon, 1970) and that antipsychotic compounds have dose-related DA receptor antagonist features (Creese et al., 1976). Clinical efficacy of neuroleptics was selectively related to the blockade of DRD2 in contrast to their effect on any other neurotransmitter system (Peroutka & Snyder, 1980). Progress in functional neuroimaging techniques has allowed the justification of the *in vitro* results under *in vivo* circumstances and the refinement of the DA hypothesis as well. The SPECT method using eligible ligands labeled with radio-isotopes such as ^{123}I-IBZM ([(*S*)-2-hydroxy-3-iodo-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide]) which binds selectively to striatal DRD2 (Kung et al., 1990), is suitable for the visualization of the mechanism of antipsychotic effects. This method is also suitable for the exploration of the functional disturbances of the DA system which may play a key role in the etiology of schizophrenia. According to the modified DA hypothesis, the elements of the DA system simultaneously show hyper-, and hypoactivity in the schizophrenic brain. Pathologically low activity in the prefrontal cortex is responsible for the cognitive deficit and, at the same time, this causes the mesolimbic-striatal hyperactivity (Davis et al., 1991). Regulation of the striatal DA release has two components (Grace, 1991). The sustained or tonic mechanism mediates the background DA activity, which is mainly modulated by glutamatergic projections from the prefrontal cortex, while the phasic component refers to action potentials of DA neurons in response to environmental stimuli (Byne & Davis, 1999). During the long-term course of schizophrenia, a chronic recurrence of hyperresponsive states of subcortical DA systems may occur which expires during remission. In these sensitive states, DA neurons are hyperreactive to different environmental stimuli and even moderate level of stress could lead to relapse of psychotic symptoms. Hyperresponsivity is associated with exaggerated DA release. During ^{123}I-IBZM SPECT measurements, the increased amount of endogenous DA leads to a decrease in isotope activity as a consequence of competition between the labeled radioligand and DA

(Laruelle, 2000). Thus, acute periods of the illness are related to the hyperactivity of the limbic-striatal DA system, which is restored by DA receptor blockade caused by antipsychotic treatment. Several positron emission tomography (PET) (Farde et al., 1989) and SPECT studies (Klemm et al., 1996) investigated the mechanism of antipsychotic action in the brain. Neuroleptics having similarly strong DRD2 antagonist properties as their prototype chlorpromazine have been proven to be effective in the treatment of positive symptoms – delusions, hallucinations, agitation – of schizophrenia. A threshold of at least 60% of DRD2 occupancy has been postulated for clinical response (Nordström et al., 1993). However, the administration of "classical" antipsychotics, induce more or less severe extrapyramidal side effects (EPS) due to the nigrostriatal DA blockade. The neuroleptic threshold is around 75-80% occupancy of DRD2, which means that further dose-dependent saturation of receptors does not enhance the therapeutic response, but significantly increase the rate of EPS (Farde et al., 1992). EPS accompanying the treatment is often serious, sometimes irreversible event, impeding the compliance of patients and hampering their quality of life. In the clinical practice, specific scales are in use for the measurement and monitoring of these signs and as well clinical symptoms. In our study, we used the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) to score the psychopathological signs, the Simpson Angus Scale (SAS) (Simpson & Angus, 1970) for assessment of the Parkinson-like features, and the Abnormal Involuntary Movements Scale (AIMS) (Fann et al., 1977) to evaluate tardive motor disturbances.

Clozapine, a dibenzodiazepine derivate was the first antipsychotic drug having equivalent efficacy with that of classical compounds but had much lower propensity to induce EPS. In addition, clozapine has other advantages over typical antipsychotic agents, including efficacy in treatment-refractory states (Kane et al., 1988), against negative symptoms (affective blunting, alogia, avolition, and anhedonia), and certain types of cognitive deficits (Meltzer & McGurk, 1999). Neuroimaging studies revealed that at clinically effective doses clozapine induces lower DRD2 occupancy (Nordström et al., 1995). However, clozapine also has a considerable antagonistic effect on serotonin $5HT_{2A}$ receptors (Farde et al., 1994). This "atypical" receptor affinity profile which may result in its unique clinical characteristics constituted the theoretical basis of the development of the new generation of antipsychotic compounds. One member of this new class is quetiapine. Several clinical investigations have confirmed its efficacy in the treatment of positive and negative symptoms and a better tolerability than that of clozapine (Wetzel et al., 1995; Arvanitis & Miller, 1997). Its receptor profile is somewhat similar to that of clozapine: in a wide range of dose quetiapine induces

only 24-28 % DRD2 occupancy (Küfferle et al., 1997) and remarkably binds to $5HT_{2A}$ receptors (Kapur et al., 2000).

2.1.2. Measurement of visual contrast sensitivity and the dopamine system

The main components of human visual information processing are the magnocellular (M) and parvocellular (P) pathways which are segregated from both functional and morphological points of view. The M pathways originated from the retina comprise large ganglion cells projecting to the magnocellular layers of the lateral geniculate nucleus and then to layer 4C alpha of the primary visual cortex. In contrast, the retinal origin of the P pathways includes small ganglion cells projecting to layer 4C beta of primary visual cortex through the parvocellular layers of the lateral geniculate nucleus. Although the M and P parallel pathways interact at the level of visual cortex, data suggest a definite functional separation even at higher levels of information processing. The M system (transient channels) is responsible for the analysis of motion and spatial location, whereas the P system (sustained channels) is related to the processing pattern and color (Bassi & Lehmkuhle, 1990). The sensitivity of the two systems is stimulus-dependent. The M system shows higher sensitivity for stimuli with low spatial frequencies (<3 cycles per degree of visual angle [c/d]) and high temporal frequencies (4-15 Hz), while the P system can be better stimulated with patterns having high spatial and low temporal frequencies (Wilson 1980). This feature allows biased investigation of each unit under certain circumstances.

Luminance contrast is the difference in luminosity of a pattern against its background. The higher the contrast between them, the easier to recognize the stimulus. With a gradual, stepwise adjustment, we are able to measure the minimal contrast necessary for perceiving a stimulus. This is contrast threshold, and its reciprocal is visual contrast sensitivity (VCS). VCS is regularly evaluated using luminance-contrast gratings with a sinusoidal luminance profile. One cycle consists of a pair of a dark bar with minimal luminance and a white bar with maximal luminance. It is possible to change both spatial and temporal parameters of stimuli within a well-defined range. Spatial frequency is the number of cycles per 1° of visual angle (c/d). In the static condition, steady patterns are used. In the dynamic condition, dark and white bars change places several times during a second. The number of changes during a second defines temporal frequency (Hz). With this method different functional units of the visual system can be investigated (Campbell, 1983) (Figure 1).

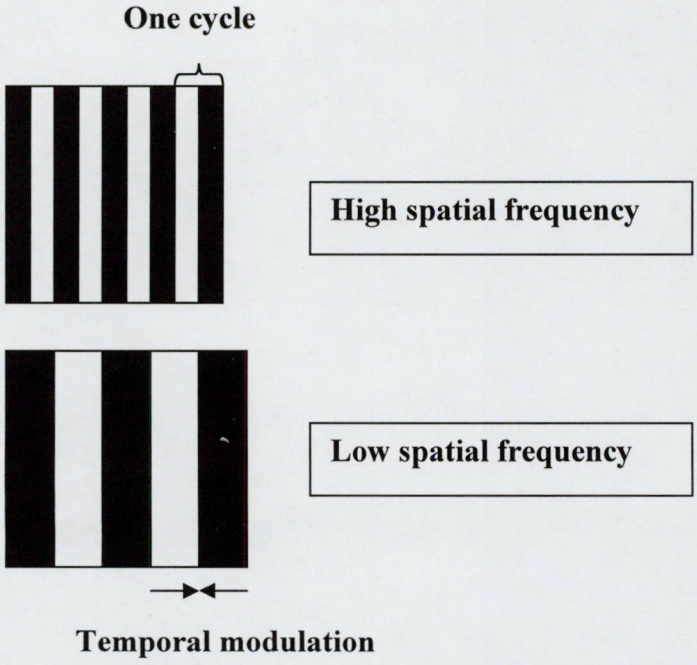
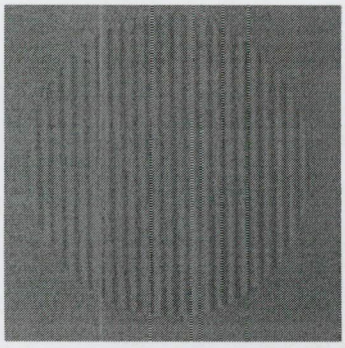
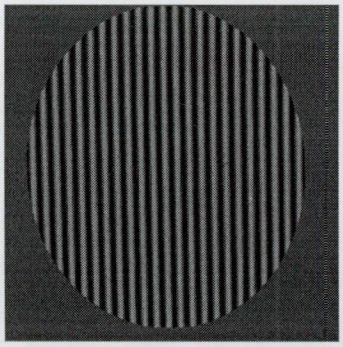


Figure 1. Spatiotemporal characteristics of visual stimuli. Brightness changes periodically to constitute dark and white bars with minimal and maximal luminance, respectively. The contrast is gradually decreased to define the minimal contrast which is indispensable for perceiving pattern. Contrast is defined by Michelson formula [$C= (L_{max} - L_{min})/ (L_{max} + L_{min})$]. Spatial frequency is the number of cycles per 1° of visual angle (c/d). Temporal frequency is the number of phase reversals during a second (Hz).



Sinusoidal grating with low contrast



Sinusoidal grating with high contrast

It has been well established that visual processing is modulated by DA (Masson et al., 1993). A hypodopaminergic state present in Parkinson's disease is accompanied by VCS reduction at spatial frequencies up to 4.8 c/d (Bodis-Wollner et al., 1987). In the dynamic condition, VCS reduction was observed mainly at low frequencies, leading to the hypothesis of transient channel dysfunction in Parkinson's disease (Mestre et al., 1990). In case of schizophrenia, the results are controversial. Some findings suggest a transient channel dysfunction (Schwartz et al., 1987), while other studies concluded that both transient and

sustained channel functions are impaired in schizophrenia (Green et al., 1994; O'Donnel et al., 1996). According to a recent study, the characteristics of VCS loss show relationship with the current symptoms. Patients with positive symptoms exhibited VCS deficit only at high spatial frequencies, whereas negative symptom patients showed reduced VCS at both low and high frequencies (Slaghuis, 1998). The results could be confounded by antipsychotic medication. Patients treated with conventional antipsychotics show parkinsonian VCS deficits related to the DA blockade (Bulens et al., 1989). The extent of impairment correlates with the daily dose of antipsychotics and with the severity of parkinsonian symptoms rated by SAS (Kéri et al., 1998). In contrast, patients receiving the atypical antipsychotic olanzapine, which has a more favorable EPS profile, show intact VCS values over the whole spatial frequency range (Kéri et al., 1999). According to these findings, it may be hypothesized that hypodopaminergic states induced by pharmacogenic DA blockade or related to the negative symptoms of schizophrenia are associated with parkinsonian VCS impairment.

Objective of the thesis: to study the relations between EPS measured by clinical rating scales, values of VCS and striatal DRD2 occupancy rates investigated by ^{123}I-IBZM SPECT.

2.2. Gene polymorphisms in schizophrenia

2.2.1. Genetic research

It is well established that schizophrenia has a hereditary component. Decades of research on genetic factors underlying schizophrenia revealed the polygenic manner of heritability, which means that susceptibility to the disease is linked to the interactions of several genes with small effect (McGuffin et al., 1995). Both linkage and association strategies have been applied in the search of genes that cause or predispose to schizophrenia. The basic principle of linkage approach is that attached transmission of neighboring genes is more probable than that of distant ones. Known genetic markers are inherited together with diseases and that refers to the localization of susceptibility genes. International linkage studies involving different samples have provided inconsistent results. Numerous genomic regions have been suspected to carry the predisposing genes: 6p24-22, 8p22-21, 22q11-12, 13q14.1-q32, 5q21-q31, 18p22-21, and 10p15-p11 (Schwab et al., 2000), but convincing evidence is still lacking (Owen, 2000). In contrast to linkage analysis, association approaches are based on the comparison of allelic frequencies of candidate genes in groups of patients and controls, and examine variants of suspected genes selected on the basis of their relevance to the putative

etiology and pathophysiology or other disease specific feature (Schwab et al., 2003). Many association studies have been carried out relying on the results of neuropharmacological and neurochemical investigations (Janka, 1995). Thus, genes encoding DA and 5-HT receptors became the main target for association studies. Some inconsistent associations have been proven between schizophrenia and the Ser9Gly polymorphism of the dopamine D3 receptor (DRD3) (Crocq et al., 1992; Spurlock et al., 1998) similarly to the T102C variant of the serotonin 5-HT_{2A} receptor (Williams et al., 1996). Another group of candidate genes play a role in the development of nervous system. Polymorphism of these genes may contribute to the maldevelopment of neurons, synapses, and receptors.

Polymorphisms may have diverse functional consequences, depending on the affected DNA site. For example, substitution in the amino acid sequence of a receptor may change the binding properties or association with intracellular signal processes. Localization in the promoter or enhancer region of a gene may lead to the modification of expression. Finally, the substitution of a nucleotide may be apparently silent but could change the stability of mRNA.

2.2.2. Polymorphisms of dopamine D3 receptor and dopamine transporter genes

The DRD3 is present in various areas of the central nervous system, including the limbic system (particularly the nucleus accumbens), the neocortex, and the cerebellum (Kerwin & Owen, 1999). It has a high affinity to both conventional and atypical antipsychotics, which also elevate the mRNA of DRD3 (Buckland et al., 1993). A recent study found 2-fold elevation in the mRNA level of the DRD3 in lymphocytes of schizophrenic patients (Ilani et al., 2001). The DRD3 gene is located on chromosome 3q13.3. Its Ser9Gly polymorphism, close to the N-terminal of the protein, has been widely studied. The homozygote variants have twice higher affinity to DA than heterozygotes (Lundstrom & Turpin, 1996). Several case-control studies found association between schizophrenia and homozygosity with a special reference to the Ser9Ser homozygote genotype (Crocq et al., 1992; Shaikh et al., 1996; Ebstein et al., 1997; Spurlock et al., 1998). However, negative results have also been published (Malhotra et al., 1998; Virgos et al., 2001). According to recent meta-analyses, the DRD3 Ser9Gly polymorphism confers weak but significant association with schizophrenia (Williams et al., 1998; Dubertret et al., 1998; Schwartz et al., 2000). The Gly9Gly homozygote variant has been found to be associated with tardive

dyskinesia (Steen et al., 1997), akathisia (Eichhammer et al., 2000), and spontaneous dyskinesia in drug-naïve schizophrenic patients (Lovlie et al., 2001).

Several pharmacogenetic studies have investigated the relationship between Ser9Gly polymorphism and therapeutic response to clozapine. Shaikh et al. (1996) found that Ser9 allele was significantly more frequent in patients than in controls (p=0.004). Both groups were of Caucasian origin. Regarding to response to treatment with clozapine (the criterion of responders was at least 20-point improvement in the Global Assessment Scale (GAF), the Ser9Ser genotype was more frequent among the non-responders (p=0.04) (Shaikh et al., 1996). Another study conducted in Pakistani patients provided similar results: a better treatment response was related to the Gly9 allele (p=0.0058) and genotypes consisting of Gly9 (Scharfetter et al., 1999). However, Malhotra et al. (1998) failed to replicate these results. They could confirm the association neither between excess DRD3 homozygosity and schizophrenia, nor DRD3 homozygosity and response to clozapine (Malhotra et al., 1998). One study suggested that the Gly9Gly genotype showed a trend toward an excess among non-responders patients receiving typical neuroleptics (Joober et al., 2000).

The dopamine transporter (DAT) eliminates released synaptic DA in the striatal regions, playing an important role in the regulation of subcortical DA neurotransmission. On the basis of findings published so far, there is no evidence of association between the 9/10 40-bp variable number of tandem repeats (VNTR) polymorphism of the DAT and schizophrenia (Li et al., 1994; Maier et al., 1996; Inada et al., 1996).

Cognitive impairment is an essential component of the phenomenology of schizophrenia. The most severely affected functions are executive and memory domains (Callicott et al., 2000; Gold et al., 1997). Executive functions include the utilization of information stored in working memory, planning, and flexible change of strategies basing on feedback. The Wisconsin Card Sorting Test (WCST) is widely used for the investigation of executive functions. During the test, cards depicting geometrical figures must be categorized according to their color, shape and number, consecutively. The criterion of completion of a category is 10 successive correct answers. Following this the investigator changes the rule of sorting without any warning, for example from color to shape. After the feedback of wrong choice, the participant should shift to the new categorization rule. Schizophrenic patients complete fewer categories than unaffected controls and fail to shift strategy: they continue to sort the cards according to the previous rule and are inclined to repeat false choices. Executive functions are related to the dorsolateral prefrontal cortex (DLPFC). In case of schizophrenia, a relationship has been demonstrated between impaired DLPFC activation and poor

performance on executive tasks (Weinberger et al., 1986; Bertolino et al., 2003). The structural bases of explicit memory functions are the temporo-hippocampal circuits, whereas implicit memory is housed in neocortical and striatal units. While implicit memory seems to be relatively preserved, deficits of explicit memory functions and also both macroscopic and microscopic impairments of temporal lobes and hippocampal structures are well established feature of schizophrenia. These alterations have been proven by neuropsychological and neuroimaging investigations (Friston et al., 1992; Andreasen et al., 1995; Lawrie & Abukmeil, 1998; Kéri et al., 2000). These disturbances can be conceptualized as a failure of cognitive control which may be due to impairments in representing, maintaining, and updating context information. Dysfunctional interactions between the malfunctioning DA system and the prefrontal cortex can lead to abnormal gating of stimuli into the DLPFC producing such cognitive disturbances (Braver et al., 1999).

Several studies have demonstrated the effect of different elements of DA system on cognitive functions. A Val158Met functional polymorphism of the catechol-*O*-methyltransferase (COMT) gene has been shown to affect executive performance and the physiology of the prefrontal cortex by influencing prefrontal dopamine signaling. The COMT valine allele is associated with relatively poor prefrontal function and may increase the risk for schizophrenia (Egan et al., 2001). It has been also demonstrated that elevated density of the dopamine D1 receptors in DLPFC of schizophrenic patients, which seems to be compensatory upregulation secondary to sustained deficiency in mesocortical DA function, correlated with poorer working memory performance (Abi-Dargham et al., 2002). Conventional antipsychotics having remarkable DRD2 antagonist effect can further worsen cognitive functions, but atypicals with high 5-HT/DA affinity ratio seem to improve verbal learning abilities, working memory, and visuo-motor functions (Keefe et al., 1999; Gallhofer et al., 1999).

Objective of the thesis: to investigate the effects of DRD3 Ser9Gly and DAT VNTR polymorphisms on therapeutic response to atypical antipsychotics as well as on cognitive functions assessed by WCST and verbal memory performance in schizophrenia.

2.2.3. Brain-derived neurotrophic factor gene polymorphism

According to the neurodevelopmental hypothesis of schizophrenia, abnormal events during early phases of development lead to altered differentiation, migration, and survival of

neurons (Weinberger, 1987). This early neurodevelopmental theory supposes that further specific developmental events, which occur during subsequent decades, make interactions with prenatal maldevelopmental processes creating manifestations of the disease. The late neurodevelopmental hypothesis focuses on the role of abnormal synaptic elimination appearing in late adolescence (Tényi & Trixler, 1999). Eventually, changes in synaptic transmission in specific neural circuits result in impaired morphology and physiology, leading to different pathologic process (*e.g.* pathologic pruning), which manifest in the clinical onset of the disease (Mirnics et al., 2001). Many researches have been focused on neurotrophic factors due to their widespread role in modulating many kinds of different developmental events (Arnold & Rioux, 2001). These proteins are the molecular regulators of synaptic plasticity, turning neuronal activity to functional and structural changes (McAllister et al., 1999). Therefore, their genetic variants with changed functions may result in an increased risk for abnormal developmental processes. Within the family of neurotrophic factors, brain-derived neurotrophic factor (BDNF) is the most predominantly expressed in the human postnatal brain, with the highest mRNA levels in neocortex and hippocampal formations (Wetmore et al., 1990). The level of BDNF mRNA shows characteristic changes during postnatal development: it increases one-third from infancy to adulthood, a period believed to be critical regarding both neuronal maturation and onset of schizophrenia (Webster et al., 2002). BDNF influences the growth of almost each central neurotransmitter system, stimulates cholinergic (Knipper et al., 1994) and glutamatergic transmission (Takei et al., 1997), promotes the survival of basal forebrain cholinergic neurons, ventral mesencephalic dopaminergic neurons, and serotonin neurons (Mamounas et al., 2000; Hyman et al., 1991). According to recently published data, BDNF regulates the expression of DRD3 in the nucleus accumbens through DA and BDNF secreting neurons, originated from ventral tegmental area (Guillin et al., 2001).

Post mortem studies have found elevated levels of BDNF in the anterior cingulate cortex and hippocampus of schizophrenic patients (Takahashi et al., 2000). Others showed increased BDNF concentrations in cortical areas and a significant decrease of this neurotrophin in the hippocampus of patients (Durany et al., 2001). BDNF levels were significantly reduced in the serum of schizophrenic patients (Toyooka et al., 2002). Neonatal ventral hippocampal lesion as an animal model of the neurodevelopmental hypothesis suppressed BDNF mRNA expression in the dentate gyrus and tended to reduce its expression in the prefrontal cortex (Lipska et al., 2001). Animals with neonatal ibotenic acid lesions of the ventral hippocampus have reduced basal levels of BDNF mRNA (Ashe et al., 2002).

The BDNF gene is located on chromosome 11p13-p14. Several polymorphisms of BDNF gene are known. Studies searching for an association between the dinucleotide repeat polymorphism (166-221 bp) and schizophrenia provided negative results (Hawi et al., 1998; Virgos et al., 2001). However, an excess of the 172-176 bp alleles was found in patients with late onset, in neuroleptic-responding patients and in non-substance-abusing patients (Krebs et al., 2000). The investigation of the Val66Met polymorphism of the BDNF gene also failed to show association with schizophrenia (Egan et al., 2003). However, a recent family-based study including a larger sample revealed significant association between BDNF polymorphism and schizophrenia (Muglia et al., 2003).

A recently identified single nucleotide polymorphism of the BDNF gene is a C270T substitution in the 5'-noncoding region in which the T270 is the mutated type. The T270 has been found to be significantly more frequent in patients with late-onset Alzheimer disease than in age-matched healthy controls (Kunugi et al., 2001).

Objective of the thesis: to analyze a putative relationship between BDNF C270T polymorphism and schizophrenia, and to compare the clinical and psychosocial characteristics of patients with different genotypes.

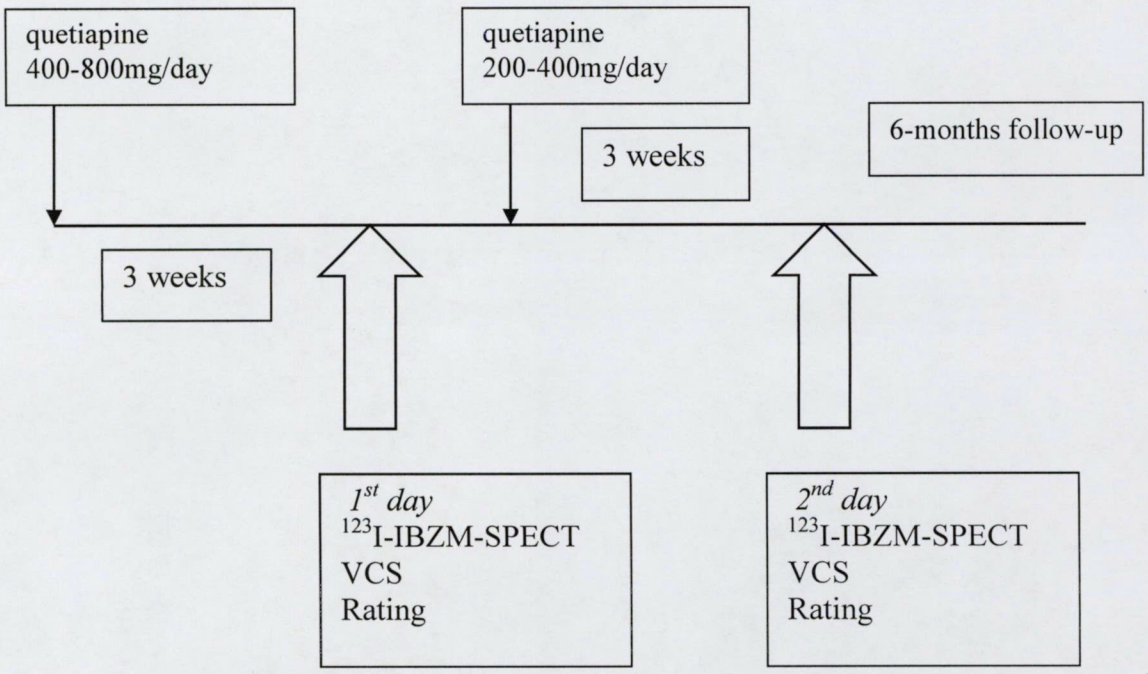
3. Subjects and methods

3.1. Receptor functions

3.1.1. Schedule of experiment

We performed two sets of experiments in schizophrenic patients receiving quetiapine. The treatment of the patients started after their admission to the inpatient unit of the Psychiatric Department due to their acutely ill state. The first examination was performed after the stabilization of their state following at least 3 weeks of monotherapy with quetiapine on constant dose ("high dose" condition). The second measurement was carried out when the patients had been continuously receiving the constant maintenance dose of quetiapine at least for 3 weeks. The maintenance dose was achieved by tapering the higher daily dose to a lower dose which fit better to the stable clinical state. Each experiment included clinical examination, measurement of VCS, and ^{123}I-IBZM SPECT procedure. The trial was accomplished by a 6 months follow-up period (Figure 2).

Figure 2. Schedule of investigation of dopamine D2 receptor functions in schizophrenia



3.1.2. Subjects

Ten patients (7 women, 3 men) with DSM-IV diagnosis of schizophrenia participated in the study. Their age was 34 ±7 years (mean ±SD). The patients were recruited from the inpatient unit of the Department of Psychiatry, University of Szeged. Written informed consent was obtained from each subject before enrolment. All subjects received a thorough psychiatric, medical and neurological evaluation, including brain CT to rule out structural lesions. All patients were free of active medical problems or substance abuse. None of the patients had been treated with long-acting depot neuroleptic medications during at last a 3 month-period before the study. The PANSS was used to evaluate the clinical symptoms. Extrapyramidal signs were rated by SZTE Egyetemi Könyvtár SZEGED 14.

Table 2. Demographical and clinical data of schizophrenia patients

Patient			**PANSS**[1]				**Interval to relapse (weeks)**	**Quetiapine dose (mg/day)**		**SAS**[2]		**AIMS**[3]	
Number	Gender	Age	1st	2nd	changes	relapse		1st	2nd	1st	2nd	1st	2nd
1.	F	45	74	74	0	-	-	400	200	10	10	0	0
2.	F	39	93	96	3	-	-	800	400	16	16	11	12
3.	F	30	52	56	4	110	5	400	200	10	10	1	1
4.	M	27	98	86	12	-	-	600	300	10	10	0	0
5.	M	30	114	108	-6	111	14	800	400	14	13	0	0
6.	F	34	53	55	2	88	35	400	200	10	10	0	0
7.	F	38	67	46	-21	98	11	600	300	10	10	4	3
8.	F	35	65	65	0	128	20	600	300	13	11	0	0
9.	F	43	82	76	-6	-	-	600	300	10	10	0	0
10.	M	23	64	55	-9	-	-	600	300	10	10	0	0

[1]Positive and Negative Syndrome Scale for Schizophrenia, [2]Simpson-Angus Scale, [3]Abnormal Involuntary Movement Scale

3.1.3. Visual contrast sensitivity procedure

Visual patterns were generated by using a standard Venus system (Neuroscientific Corporation, USA). Stimuli were vertical luminance-contrast gratings with sinusoidal luminance profile. Two temporal frequencies (0 Hz in the static test, 8 Hz in the dynamic test) and 9 spatial frequencies (0.5, 1.2, 1.9, 2.9, 3.6, 4.8, 5.7, 7.2, and 14.4 c/d) were included. The stimulus display subtended 13° x 13° from a viewing distance of 1 m. A trial consisted of two consecutive observation periods, each initiated by a brief tone. The duration of an observation period was 1 s. The grating was presented randomly either in the first or the second observation period immediately after the initiating tone. The subject's task was to indicate whether the stimulus appeared after the first or second tone by pressing one of the two response buttons on a separate response pad. The exposure time of the gratings was 500 ms. Responses were accepted from the onset of a stimulus up to 10 s after the completion of the trial. The next trial was not initiated without a response. At the beginning, the contrast was set at 10 dB above the normal values. The computer automatically decreased or increased the contrast by 3 dB when the subject gave, respectively, 2 right or 2 wrong consecutive responses

at a given spatial frequency. If a pair of correct or wrong responses was given, the computer repeated the measurement without the modification of the contrast level. The contrast threshold was the minimal contrast level at which subjects were able to give 2 consecutive correct responses. Analysis of variance (ANOVA) was used for statistical analysis of VCS values received at different dose levels of quetiapine.

3.1.4. SPECT procedure

During the SPECT procedure, 60 minutes after oral administration of 450 mg potassium-perchlorate, the patient was given 185 MBq ^{123}I-IBZM (Cygne BV, Holland) intravenously. The SPECT data acquisition was started 90 minutes after the IBZM injection. For this a rotating single head gamma camera with low energy high-resolution collimator (Siemens Diacam) connected to a computer system (Siemens Icon) was used. Data were collected for 120 projections (360° rotation) in 128x128 matrix. The acquisition times were 30 seconds per projection. Images were reconstructed by filtered backprojection using a Butterworth filter with a cut-off frequency of 0.5 Nyquist, power factor 10. The reconstructed images were corrected for gamma ray attenuation using the Chang method with an attenuation coefficient of 0.12cm^{-1}.

The reconstructed slices were visually assessed by three well-trained observers and semi-quantitatively evaluated. The observers were blind to the medication status of the subject. In three consecutive transversal slices with the highest ^{123}I-IBZM uptake at the level of striatum, striatum/occipital lobe activity ratios were calculated and averaged separately on the left and right side. The ratios were calculated using region of interest (ROI) method. Elliptical ROI-s were placed on the consecutive transversal slices at the level of basal ganglia with the highest uptake of the striatum. The region size of striatum and the region size of the occipital lobe were 100 ±20 pixels and 47 ±6 pixels, respectively. Two independent nuclear medicine specialists tested the reproducibility of the method used for determining the ratio. The ratios calculated by the two evaluators were statistically not different and correlated well (p=0.053).

For the evaluation of the experimental data, statistical analysis was performed by two-tailed t-test and correlations were calculated by Pearson method. The significance level was set at p<0.01.

3.2. Dopamine D3 receptor Ser9Gly polymorphism and dopamine transporter variable number of tandem repeats (VNTR) polymorphism

3.2.1. Subjects

Altogether 120 Caucasian volunteers participated in the study. Seventy-five of them (34 men and 41 women) received the DSM-IV diagnosis of schizophrenia (APA, 1994) and 45 (17 men and 28 women) were healthy controls who were recruited from the hospital staff and general population. Clinical symptoms were evaluated with the PANSS and the Global Assessment of Functioning (GAF) scale (APA, 1994). Participants also received the Mini International Neuropsychiatric Interview Plus (MINI Plus) (Balázs & Bitter, 2000). From the whole sample, 36 patients (14 men and 22 women) and 20 matched control subjects (8 men and 12 women) were included in the neuropsychological assessment (Table 3). The patients received atypical antipsychotics at least for 12 weeks (clozapine: n=5, olanzapine: n=29, quetiapine: n=16, risperidone: n=25). The treatment was initiated during an acute psychotic episode, which resulted in a substantial drop in community functions and required hospitalization. A detailed overview of medical documentation (diagnosis, symptom ratings, and medication) was carried out by an expert psychiatrist who was blind to the results of genotyping and neuropsychological assessment. In addition, each patient was interviewed individually. A patient was considered responder if there was an improvement of 20 points or more in the GAF scale during the treatment period. All participants gave their informed consent.

3.2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes. In the case of DRD3, the polymerase chain reaction (PCR) (Progene, Techne Cambridge) was carried out with the following primers: 5' – GCT CTA TCT CCA ACT CCT ACA – 3' and 5' – AAG TCT ACT CAC CTC CAG GTA – 3'. 100 ng of genomic DNA was amplified in 25 μl reaction mixture [1.5 mM $MgCl_2$ (Boehringer Mannheim), 100 μM of each dNTP (dATP, dCTP, dGTP, dTTP) (Boehringer Mannheim), 5 pM of each primer (Creative Lab), 0.5 mM dithiothreitol (Sigma), 0.01% gelatin (Sigma), 1.5 U Taq polymerase (Sigma)]. The PCR protocol included a

denaturation of DNA (95°C for 6 min) and 35 cycles with the following steps: 92°C for 1 min, 56°C for 1 min, 72°C for 1 min. The final extension was 72°C for 8 min. The PCR product was digested with 1 U MscI/15 μl (GibcoBRL). The electrophoresis was carried out on 5% acrylamide/bis acrylamide gel (BioRad). In the case of DAT, PCR amplification was carried out with the following primers: DATVNTRF: 5' – TGT GGT GTA GGG AAC GGC CTG AG – 3' and DATVNTRR: 5' – CTT CCT GGA GGT CAC GGC TCA AGG – 3'. 100 ng genomic DNA was used for PCR reaction in 25 μl reaction mixture (1.3 mM $MgCl_2$, 200 μM dNTP mix, 0.25 μM of each primer, 5% DMSO, 1.5 U Taq polymerase). The PCR program included the following steps: denaturation of DNA (5 min at 94°C), 30 cycles of 30 sec at 94°C, 30 sec at 62°C, and 30 sec at 72°C. This was followed by the final extension for 10 min at 72°C. The electrophoresis was carried out on 8% acrylamide/bis acrylamide gel. In both cases, the bands were visualized by ethidium bromide (Sigma) under an UV transilluminator.

3.2.3. Neuropsychological assessment

For the assessment of executive functions, the WCST was used (Heaton et al., 1993). Mean number of categories completed, number of perseverative errors, and failure to maintain set were the dependent variables. A simple word-list learning was used for the assessment of explicit verbal memory where the number of recalled words was the dependent measure (Gur et al., 2000).

3.2.4. Data analysis

Chi square (χ^2) tests were used to evaluate the differences in allele and genotype frequencies in the case of schizophrenia patients and controls, responders and non-responders, and patients with different degree of severity of cognitive dysfunctions. Analyses of variance (ANOVAs) were used to compare the neuropsychological performance of the patients with different genotypes. For *post hoc* comparisons, data were treated with Scheffé's tests. Clinical and demographic data were compared with two-tailed *t*-tests. The genotype frequencies were investigated with Fisher's exact test to test for Hardy-Weinberg equilibrium.

3.3. Brain-derived neurotrophic factor C270T polymorphism

3.3.1. Subjects

A total of 169 Caucasian volunteers participated in the study. Hundred and one of them (62 men and 39 women) received the DSM-IV diagnosis of schizophrenia. Their age was 39.8 ±11.8 years (mean ±SD). Sixty eight (39 men and 29 women) of them were healthy controls, age was 36.8 ±11.1 years (mean ±SD). Controls were recruited from the hospital staff and general population. Clinical symptoms were evaluated with the PANSS and the GAF scale. Participants also received the MINI Plus. A detailed overview of medical documentation (diagnosis, symptom ratings, and medication) was carried out by a psychiatrist who was blind to the results of genotyping. In addition, each patient was interviewed individually. All participants gave their informed consent.

3.3.2 Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes. The PCR reaction (Progene, Techne Cambridge) was carried out with the following primers: 5'-CAG AGG AGC CAG CCC GGT GCG-3' and 5'-CTC CTG CAC CAA GCC CCA TTC-3'. A 100 ng sample of genomic DNA was amplified in 25 µl reaction mixture [1.1 mM $MgCl_2$ (Boehringer Mannheim), 200 µM of each dNTP (dATP, dCTP, dGTP, dTTP) (Boehringer Mannheim), 1 µM of each primer (Creative Lab), 1.5 U/25 µl Taq polymerase (Sigma)]. The PCR program included the following steps: denaturation of DNA (5 min at 94°C), 30 cycles of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C. This was followed by the final extension for 5 min at 72°C. The PCR product was digested with *Hinf*I [5U/25 µl (Gibco)]. The electrophoresis was carried out on 8% acrylamide/bis acrylamide gel (BioRad). The bands were visualized by ethidium bromide (Sigma) under an UV transilluminator.

3.3.3. Data analysis

The genotype frequencies were investigated with Fisher's exact test to test for Hardy-Weinberg equilibrium. χ^2 test was used to evaluate the differences in genotype and allele frequencies in the schizophrenia patients and controls, and to evaluate gender distributions in the two experimental groups. The normality of data distribution was checked with Kolmogorov-Smirnov test. Student's *t*-test (two-tailed) was used to compare the clinical and demographical parameters. The level of statistical significance was defined as $p<0.05$.

4. Results

4.1. Receptor functions

The PANSS scores obtained form the high or low dose conditions did not show significant difference. There were no significant dose-dependent differences in the SAS and AIMS scores (Table 2). Significant dose-related differences in VCS were not observed either in the static (Figure 3) or dynamic conditions (Figure 4).

Figure 3. Static contrast sensitivity under high and low dose conditions

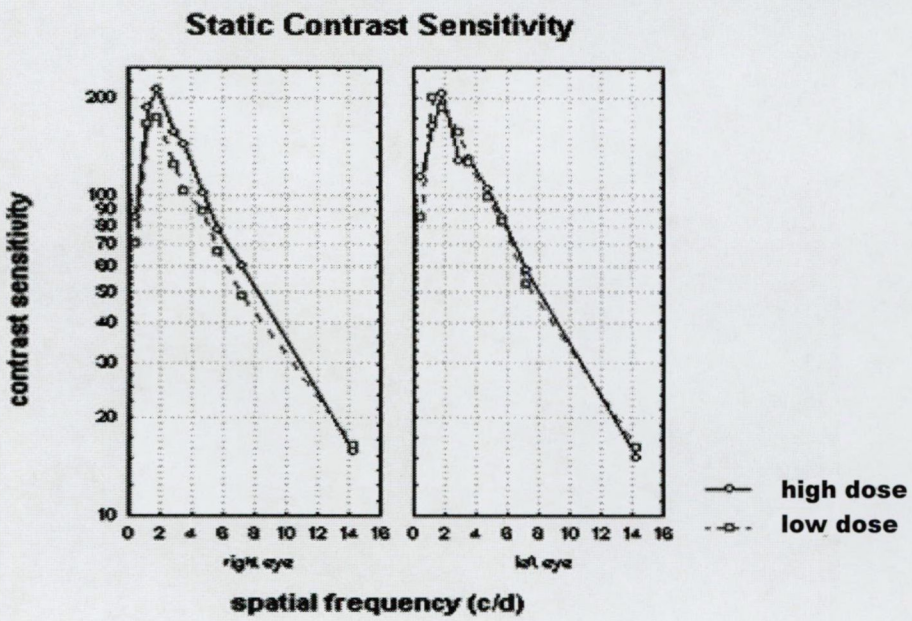
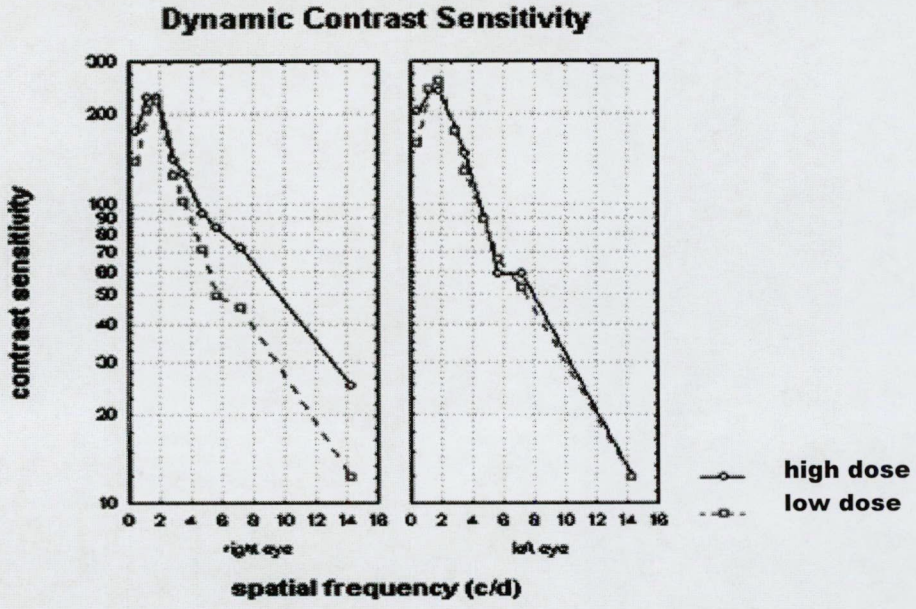


Figure 4. Dynamic contrast sensitivity under high and low dose conditions



The striatal DRD2 receptor activity was found to be suppressed at the 1st ^{123}I-IBZM SPECT investigation in 3 patients (patients No: 1, 4, 9) and normal in 7 patients by the visual evaluation (Table 3). In the 2nd investigation, contrary of decreasing the dose of quetiapine, the receptor occupancy increased in 4 (patients No: 3, 5-7), was unchanged in 3 (patients No: 2, 8, 10) and decreased in 3 patients visually. The quantitative evaluation revealed individually different striatum/occipital ratios at the 1st and also at the 2nd investigation. The changes of the striatal ^{123}I-IBZM uptake ranged from –27 to +35 %. The average uptake values of ^{123}I-IBZM statistically did not differ between the 1st SPECT and the 2nd one (1.70 ±0.23 [mean ±SD] vs. 1.68 ±0.12 [mean ±SD]). In 5 subjects the values of striatum/occipital ratio increased and in 5 persons decreased during lowering the dose of quetiapine. From the 5 patients with increased D2 receptor occupancy, each patient showed a relapse of acute schizophrenic episode which was not seen in patients with decreased receptor occupancy. We defined relapse as the worsening of psychotic symptoms which requires escalation of dose of maintenance therapy or change of antipsychotic therapy. The initial striatum/occipital ratio was significantly higher in patients with relapse compared to the others (1.86 ±0.17 [mean ±SD], 1.53 ±0.15 [mean ±SD], $p<0.01$). The D2 receptor occupancy changes correlated with the time interval until the relapse ($p<0.01$), but not with the PANSS changes or with initial ^{123}I-IBZM uptake ratios.

Table 3. Results of the ^{123}I-IBZM SPECT investigations in schizophrenic patients treated with quetiapine

Patient No.	Visual evaluation of DRD2 occupancy		Semiquantitative evaluation (striatal/occipital ratio)				
	1st day	2nd day	1st day		2nd day		Changes %
			R	L	R	L	
1.	decreased	decreasing	1.44	1.33	1.69	1.48	29
2.	normal	unchanged	1.74	1.66	1.67	1.71	1
3.*	normal	increasing	2.20	2.10	1.81	1.86	-29
4.	decreased	decreasing	1.48	1.56	1.79	1.78	35
5.*	normal	increasing	1.81	1.62	1.59	1.52	-19
6.*	normal	increasing	1.87	1.81	1.78	1.61	-15
7.*	normal	increasing	1.81	1.81	1.58	1.55	-27
8.*	normal	unchanged	1.85	1.76	1.87	1.58	-9
9.	decreased	decreasing	1.38	1.37	1.53	1.61	29
10.	normal	unchanged	1.61	1.72	1.69	1.81	5

* patients showing symptoms of relapse during follow-up

4.2. Dopamine D3 receptor Ser9Gly polymorphism and dopamine transporter VNTR polymorphism

Table 4 shows the genotype and allele frequencies of the DRD3 S/G polymorphism in responder and non-responder patients with schizophrenia and controls. First, neither any specific genotype nor a particular allele was associated with schizophrenia when compared with the controls ($p>0.3$). In contrast, the S/S genotype was more frequent among the non-responders than in the responders ($\chi^2= 9.71$, $df=1$, $p=0.0018$). A similar association was found when the S allele was taken into consideration ($\chi^2=5.68$, $df=1$, $p=0.0172$).

Table 4. Genotype and allele frequencies of the dopamine D3 receptor (DRD3) S/G polymorphism in the responder and non-responder patients with schizophrenia and controls

	S/S	S/G	G/G	S	G
Responders (n=47)	13 (0.28)	34 (0.72)	0	60 (0.64)	34 (0.36)
Non-responders (n=28)	18 (0.64) *	10 (0.36)	0	46 (0.82) **	10 (0.18)
Controls (n=45)	22 (0.49)	22 (0.49)	1 (0.02)	66 (0.73)	24 (0.27)

* p= 0.0018; ** p= 0.0172

Table 5 shows the genotype and allele frequencies of the DAT VNTR polymorphism in the responder and non-responder schizophrenia patients and controls. None of the genotypes or alleles were associated with schizophrenia (p>0.5), and none of them were more frequent in responder or non-responder patients (p>0.1).

Table 5. Genotype and allele frequencies of the dopamine transporter (DAT) VNTR polymorphism in the responder and non-responder patients with schizophrenia and controls

	10/10	10/9	9/9	10	9
Responders (n=47)	27 (0.58)	18 (0.38)	2 (0.04)	72 (0.77)	22 (0.23)
Non-responders (n=28)	12 (0.43)	13 (0.46)	3 (0.11)	37 (0.66)	19 (0.34)
Controls (n=45)	24 (0.53)	18 (0.40)	3 (0.07)	66 (0.73)	24 (0.27)

Table 6 depicts the clinical, demographical, and neuropsychological results as a function of genotypes. There was no significant difference among the subgroups in age, education, PANSS positive, negative, global symptoms, and GAF scores (t-test, p>0.1). An ANOVA

comparing the patients with S/S and S/G genotypes (n=36) and the controls (n=20) for the WCST categories completed indicated a significant main effect ($F(2,53)=20.58$, $p<0.0001$).

Table 6. Clinical, demographical, and neuropsychological characteristics of the patients of schizophrenia with different genotypes and control subjects

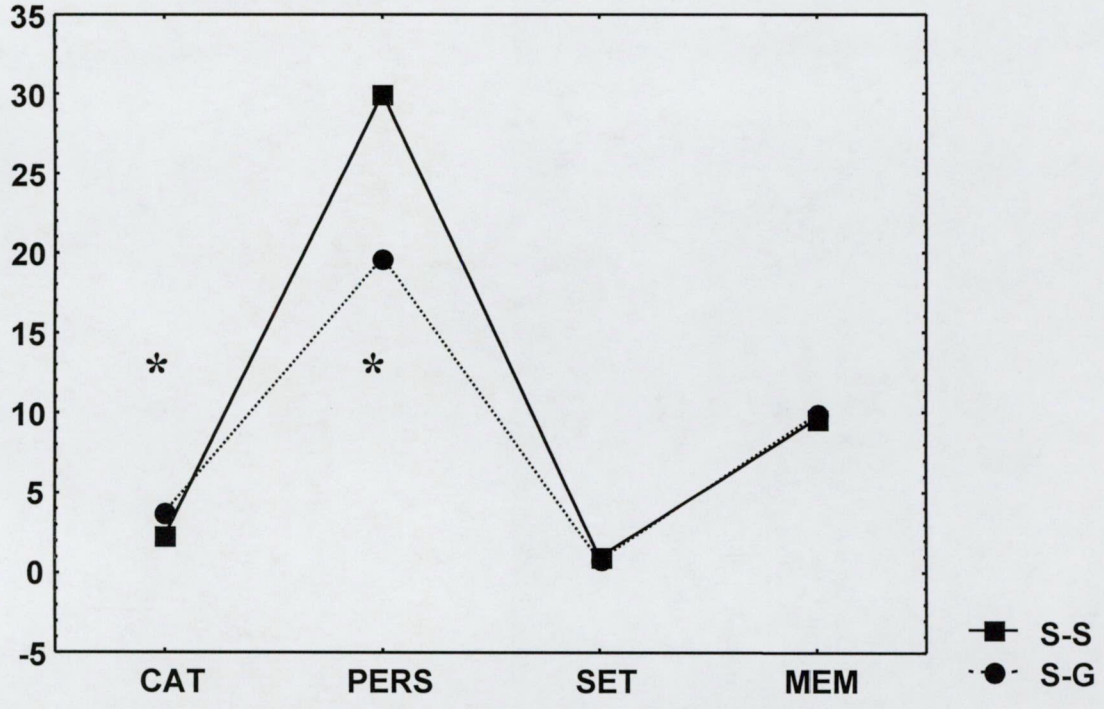
	Controls	DRD3 S/S	DRD3 S/G	DAT 10/10	DAT 9/10
Age (years)	36.4 (12.8)	37.6 (9.7)	38.1 (11.8)	34.5 (9.8)	35.8 (11.6)
Education (years)	10.0 (2.4)	10.9 (3.5)	10.9 (2.6)	11.0 (2.9)	11.0 (3.2)
WCST cat	5.2 (1.0)	2.3 (1.1)	3.8 (1.7)	3.4 (1.7)	3.2 (1.6)
WCST pers	9.2 (4.7)	30.0 (10.6)	19.7 (12.0)	21.1 (12.5)	25.6 (11.7)
WCST set	0.7 (0.3)	1.0 (0.5)	0.8 (0.4)	0.8 (0.5)	1.0 (0.6)
VR	15.7 (3.7)	9.6 (3.7)	9.9 (5.2)	9.8 (4.5)	9.3 (5.0)
GAF	-	47.3 (19.4)	53.2 (16.9)	51.6 (19.1)	49.8 (18.0)
PANSS P	-	20.2 (7.8)	20.6 (8.4)	21.4 (8.0)	19.5 (8.3)
PANSS N	-	24.4 (7.9)	24.1 (6.0)	24.8 (6.8)	23.7 (7.3)
PANSS G	-	46.4 (13.6)	46.2 (15.0)	48.3 (14.6)	44.3 (14.4)

Data are mean (standard deviation). DRD3 – dopamine D3 receptor, DAT – dopamine transporter, WCST – Wisconsin Card Sorting Test, cat – categories completed, pers – number of perseverative errors, set – failure of maintaining a set, VR – verbal recall, GAF – Global Assessment of Functioning, PANSS – Positive and Negative Syndrome Scale, P – positive symptoms, N – negative symptoms, G – general symptoms

Both schizophrenia subgroups completed fewer categories in comparison with the controls ($p<0.005$). In addition, the patients with S/S genotype completed fewer categories than the patients with S/G genotype ($p<0.01$). Similar results were obtained for the WCST perseverative errors (main effect of group: $F(2,53)=20.58$, $p<0.0001$; controls vs. schizophrenia: $p<0.005$; S/S vs. S/G: $p<0.01$). In contrast, no group effect was found in the case of WCST failure to maintain set ($p>0.1$). Finally, in the case of verbal recall

schizophrenia patients again showed an impaired performance (main effect of group: $F(2,53)=14.76$, $p<0.0001$; *post hoc* comparison: $p<0.0005$), but the S/S and S/G subgroups did not differ significantly ($p>0.5$) (Figure 5). Homologous analyses for the DAT genotypes confirmed that the patients with schizophrenia were impaired irrespective to the VNTR polymorphism ($p<0.005$). We found no significant difference between the subgroups with 10/10 and 9/10 genotypes. Overall, there was no significant difference between the performance of male and female participants ($p>0.5$).

Figure 5. Gene effect of DRD3 Ser9Gly polymorphism on executive and explicit memory functions in schizophrenic patients



CAT - categories completed, PERS - number of perseverative errors, SET – failure of maintaining set, MEM – explicite memory, S-S – Ser9Ser genotype, S-G – Ser9Gly genotype
*=$p<0.01$

To further elucidate the significant difference between the S/S and S/G schizophrenia patients, the participants of these subgroups were divided according to the severity of cognitive dysfunctions. In the case of WCST categories, 20 patients showed severe impairment (>2 SD below the control mean) from which 13 had S/S and 7 had S/G genotype. In the moderately impaired group (n=8) (1-2 SD below the control mean), 2 patients had SS and 6 patients had SG genotype. Most notably, in the remainder well-performing group (n=8) (<1 SD below the control mean) each subject was characterized with S/G alleles. Thus, the

S/S genotype was over-represented in the most severely affected group in comparison with the moderately affected group (χ^2=3.63, *df*=1, *p*=0.0552) and with the well-performing schizophrenia patients (χ^2=9.31, *df*=1, *p*=0.0013). Analogous analyses concerning the WCST perseverative errors yielded similar results (severely impaired (n=18): 12 S/S, 6 S/G; moderately impaired (n=12): 3 S/S, 9 S/G; well-performing (n=6): 0 S/S, 6 S/G). Again, the S/S genotype was more frequent in the patients with the worst performance compared with the moderately dysfunctional group (χ^2=5.00, *df*=1, *p*=0.0254) and with the well-performing patients (χ^2=8.60, *df*=1, *p*=0.0047). In the case of explicit memory, a similar portion of patients showed severe disturbances as in the WCST (n=19), but the distribution of S/S and S/G genotypes was more balanced (8 S/S and 11 S/G), and did not differ significantly from the moderately impaired (n=7; 3 S/S, 4 S/G) and well-performing (n=10; 4 S/S, 6 S/G) subgroups (p>0.5).

4.3. Brain-derived neurotrophic factor C270T polymorphism

The genotype distributions for the patient and control groups were not significantly deviated from the Hardy-Weinberg equilibrium. Regarding the genotype frequency, the C/T genotype was overrepresented in the schizophrenia patients (25.7%) compared with the controls (5.9%) (χ^2=11.17, *df*=1, *p*=0.0008). Similarly, the 270T allele was more frequent in the patients (13.9%) than in the controls (2.9%) (χ^2=11.31, *df*=1, *p*=0.0008) (Table 7).

Table 7. Genotype and allele frequencies of the brain-derived neurotrophic factor (BDNF) C270T polymorphism in patients with schizophrenia and controls

	Genotype			**Alleles**	
	C/C	**T/C**	**T/T**	**C**	**T**
Patients n=101	74 (73.3%)	26 (25.7%)*	1 (1.0%)	174 (86.1%)	28 (13.9%)*
Controls n=68	64 (94.1%)	4 (5.9%)	0 (0%)	132 (97.1%)	4 (2.9%)

* *p*=0.0008

In the patient group, the mean value for PANSS positive symptoms was 19.4 (S.D.=8.2), for negative symptoms, 24.1 (S.D.=8.4), and for global symptoms, 46.5 (S.D.=17.9). The mean GAF score of the patients was 44.2 (S.D.=17.9). There was no significant difference in PANSS items and GAF scores between the patients with C/C and C/T genotypes ($p>0.4$, t-test). The patient and the controls did not differ in age ($p=0.10$, t-test). Similarly, the gender distribution was similar in both groups ($p=0.60$, χ^2 test).

5. Discussion

5.1. Receptor functions

Our results from clinical examination (extrapyramidal scales), ^{123}I-IBZM SPECT investigation, and VCS measurement uniformly proved that the atypical antipsychotic quetiapine does not induce pronounced DRD2 blockade even when higher doses are administered. It is a well-known view that DRD2 blockade modulates the antipsychotic effect of treatment (Klemm et al., 1996) and neuroleptic induced extrapyramidal symptoms are also quantitatively related to DRD2 occupancy (Farde et al., 1992). Accordingly, the receptor binding propensity of an antipsychotic agent is a reliable predictor of its potential to induce extrapyramidal symptoms (Tauscher et al., 2002). Classical antipsychotics cause a higher DA blockade even at lower doses, while atypical compounds tend to generate only low striatal DRD2 blockade. Other atypicals like risperidone or olanzapine may cause DRD2 occupancy above 75 % in a dose dependent manner; however the frequency of extrapyramidal symptoms remains rare. It could be explained by the high $5HT_2$/DA affinity ratio which attributes almost every novel antipsychotic: the blockade of serotonin receptors could enhance DA transmission and thus decreases the risk of extrapyramidal symptoms (Meltzer, 1995).

According to a recently published hypothesis, the advantageous effect/side effect profile of atypical antipsychotics can be explained by a common mechanism. These drugs can be characterized by only transiently high DRD2 occupancy and then a fast dissociation from the receptor (Kapur & Remington 2001). In this context, different VCS results from studies including patients who received different antipsychotic agents could be well explained. Drugs with high DRD2 affinity cause reduced VCS as it can be observed in patients with Parkinson's disease, but atypical antipsychotics do not have such detrimental effects (Kéri et al., 1999). Although VCS is influenced by many factors (optic properties of the eye, photoreceptor

functions, age, attention etc.), we consistently found that dopaminergic status of the visual system is a major determining factor (Kéri et al., 2000, 2002). The site of action is dubious; the retina and the lateral geniculate nucleus are possible candidates.

In the majority of our cases, after the introduction of quetiapine treatment, the first ^{123}I-IBZM SPECT investigation showed low striatal DRD2 occupancy. During the observation period, in accordance with the low D2 receptor occupancy, none of the patients exhibited extrapyramidal symptoms. During the 6 months follow-up we observed clinical relapse in 5 patients. Each of them showed decrease in S/O ratio in spite of dose decrease.

The different levels of D2 receptor occupancy and receptor occupancy changes might depend on the individual dopaminergic status of the patients. In schizophrenia the dysregulation of dopamine system includes the lower level of tonic DA release and an exaggerated DA response to different stimuli such as stress and psychoactive substances (Weinberger et al., 1987; Grace, 1991). These subcortical anomalies show relationship with structural and functional impairments of the dorsolateral prefrontal cortex (Bertolino et al., 2000). This complex disturbance of regulation is specific to active phases of the illness, including prodromal phase, psychotic states and subsequent relapses, which could last several months (Laruelle et al., 1999). The patient with actually stable state of illness does not show this neurochemical feature. In the active phase, stimuli can elicit increased dopamine release in the striatum, leading to a decrease in the S/O ratio during ^{123}I-IBZM SPECT investigation (Laruelle, 2000). At the time of the 2nd investigation, all of our patients were in a clinically stable state. In patients showing relapse of symptoms during the follow-up phase, the persistent hyperresponsive state of the striatal dopaminergic system might explain the increase in receptor occupancy in spite of the decreased dose of antipsychotic medication, delineating a group of patients with a higher risk for relapse. Of course, this finding must be interpreted cautiously because of the small sample size.

5.2. Dopamine D3 receptor Ser9Gly polymorphism and dopamine transporter VNTR polymorphism

We investigated the distribution of DRD3 Ser9Gly and DAT VNTR polymorphisms between groups and the effect of these variants on therapeutic response and cognitive functions. We found the Ser9 allele and Ser9Ser genotype to be significantly more frequent among non-responders. The patients with Ser9Ser genotype showed poorer executive

performance than Ser9Gly genotype ones. We failed to show any association regarding the DAT VNTR variants. Our results referring to the effect of Ser9Gly polymorphism on therapeutic response are in congruence with two previous trials investigating patients on clozapine therapy (Shaikh et al., 1996; Scharfetter et al., 1999), but are more robust which may be due to the inclusion of patients receiving other atypical antipsychotics. These compounds are different in their pharmacodynamic and clinical properties due to their multireceptorial action, which could lead to more pronounced association with a poorer treatment response when a particular DRD3 variant is present.

The association of poorer WCST performance with DRD3 Ser9Ser genotype in schizophrenia is of particular interest. This phenomenon was restricted to the executive components of the task. The failure to maintain set was not impaired in the patients, which suggests that they successfully stayed on task. Moreover, the patients with Ser9Ser and Ser9Gly genotypes showed statistically indistinguishable performances in the explicit memory task. This suggests two important possibilities. First, the Ser9Ser patients are not likely to display a markedly impaired WCST performance simply because of a more severe generalized cognitive deficit in comparison with the Ser9Gly patients. It is also notable that these two schizophrenia subgroups did not differ in demographic parameters and GAF/PANSS-scores, which suggests that poorer therapeutic response and executive dysfunctions are not simple consequences of a more severe psychopathology. Second, the contribution of DRD3 polymorphism to the prefrontal executive system seems to be quite specific contrasting with the hippocampal explicit memory system. The finding that patients with Ser9Ser genotype may show poorer prefrontal functions is also supported by the data of Rybakowski et al. (2001) who found that fixation and smooth pursuit eye movement disturbances were higher in schizophrenia patients with this genotype, since eye movement dysfunctions are often linked to prefrontal pathology (O'Driscoll et al., 1999). Considering another component of the dopaminergic system, Egan et al. (2001) demonstrated that the high-activity Val allele of the COMT gene predicted poorer WCST performance and less efficient prefrontal activation. The Val allele is also related to attentional impairment, slower psychomotor speed (Bilder et al., 2002), eye movement disturbances (Rybakowski et al., 2002), and abnormalities of the P300 component of event-related potentials (Gallinat et al., 2003).

Egan et al. (2001) also suggested that the DAT might play a less important role in prefrontal functions because of its low density therein. This hypothesis was confirmed by the present results, which indicated no significant WCST difference between the patients with distinct DAT genotypes.

5.3. Brain-derived neurotrophic factor C270T polymorphism

The results of this study demonstrated an association of schizophrenia and T270 variant of BDNF gene. This finding is consistent with the data of a Japanese group (Kunugi et al., 2003). BDNF is a member of trophic factors with a notably complex role in the normal development of nervous system (Thoenen 1991). One of its most important effects is the modulation of DA system: BDNF secreted by midbrain dopaminergic neurons regulates the expression of DRD3 in the nucleus accumbens and hence controls the responsiveness of these neurons to DA which is a fundamental aspect of neuronal plasticity (Guillin et al., 2001). It is presumable that genetic variants of the BDNF have altered function and effects on developmental events. For example, a single nucleotide substitution (A758G) causing Val/Met substitution at codon 66 in the BDNF gene affects intracellular distribution, packaging, and release of the BDNF protein in vitro. Furthermore, in humans, this polymorphism has significant effects on verbal episodic memory, hippocampal physiological activation, and measures of hippocampal neuronal integrity and synaptic abundance (Egan et al., 2003). These facts made BDNF to be a candidate gene for schizophrenia. Studies focusing on the dinucleotide repeat polymorphism of BDNF found negative results (Hawi et al., 1998; Krebs at el., 2000; Virgos et al., 2001; but see Muglia et al., 2003), similarly to that of investigating the Val66Met variant (Egan et al., 2003). The C270T single nucleotide substitution (Kunugi et al., 2001) has recently been reported to be more frequent in schizophrenia comparing to controls (Kunugi et al., 2003), with only weak significance in respect of mutant T270 allele ($p=0.037$) and C270T heterozygote status ($p=0.034$) as well. In our sample, the association was statistically more significant ($p=0.008$) and the heterozygote status was more common in the controls (4.5% vs. 5.9%), which can be the consequence of differences of allele distributions between races. Further studies are warranted to replicate these findings and further trials should clarify the functional consequences of the BDNF C270T polymorphism regarding the etiology and clinical characteristics of schizophrenia.

5.4. Final conclusions

We investigated certain functional and genetic aspects of central DA system in schizophrenia. Here we demonstrated the favorable effect of atypical antipsychotic quetiapine on DA system, and delineated a group of patients with higher risk for relapse using clinical rating scales, visual contrast sensitivity measurement and visualisation of striatal DRD2 occupancy. We presented relationship between cognitive impairments as well as therapeutic response and DRD3 Ser9Gly polymorphism. And last we found an association between BDNF C270T polymorphism and schizophrenia.

6. References

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8. Appendix: Papers related to the thesis